

RESEARCH NOTE

Helminth and respiratory mite lesions in Pinnipeds from Punta San Juan, Peru

Mauricio Seguel^{1*}, Karla Calderón^{2,5}, Kathleen Colegrove³, Michael Adkesson⁴,
Susana Cárdenas-Alayza⁵ and Enrique Paredes⁶

¹Department of Pathology, College of Veterinary Medicine, University of Georgia, 501 DW Brooks, Athens, GA, 30602, USA;

²Universidad Tecnológica del Perú, Lima, Peru; ³Zoological Pathology Program, College of Veterinary Medicine, University of Illinois at Urbana-Champaign, Brookfield, IL, 60513, USA; ⁴Chicago Zoological Society, Brookfield Zoo, Brookfield, IL 60513, USA;

⁵Centro para Sostenibilidad Ambiental, Universidad Peruana Cayetano Heredia, Av. Armendáriz 445, Lima 18, Perú;

⁶Instituto de Patología Animal, Facultad de Ciencias Veterinarias, Universidad Austral de Chile, Isla Teja s/n, 5090000, Valdivia, Chile

Abstract

The tissues and parasites collected from Peruvian fur seals (*Arctocephalus australis*) and South American sea lions (*Otaria byronia*) found dead at Punta San Juan, Peru were examined. The respiratory mite, *Orthohalarachne attenuata* infected 3 out of 32 examined fur seals and 3 out of 8 examined sea lions, however caused moderate to severe lymphohistiocytic pharyngitis only in fur seals. Hookworms, *Uncinaria sp.*, infected 6 of the 32 examined fur seals causing variable degrees of hemorrhagic and eosinophilic enteritis. This parasite caused the death of 2 of these pups. In fur seals and sea lions, *Corynosoma australe* and *Contracaecum osculatum* were not associated with significant tissue alterations in the intestine and stomach respectively. Respiratory mites and hookworms have the potential to cause disease and mortality among fur seals, while parasitic infections do not impact significantly the health of sea lions at Punta San Juan, Peru.

Keywords

Hookworm, mite, *Orthohalarachne spp.*, Peruvian fur seal, South American sea lion, *Uncinaria sp.*

Introduction

Marine Mammals are top predators of aquatic ecosystems and their health is highly influenced by the conditions of the marine environment (Gulland and Hall 2007). Therefore, the study of marine mammals' health is important from the ecological and one health perspectives. Among the agents of disease described in marine mammals, a large number correspond to metazoan parasites, many of them of zoonotic significance (Sepulveda *et al.* 2015). In the case of otariids (eared seals), one of the most important parasitic diseases described is hookworm infection, however the effect of these nematodes is highly variable between populations (Seguel and Gottdenker 2017). For other helminth and arthropod parasites information on the effect of these infections in the host is very limited since

most studies only survey epidemiological aspects but few investigate the potential role of these parasites as agents of disease or mortality. In the Southern hemisphere, information is even more limited given the lower number of researchers and very large marine ecosystems. In the Peruvian coast, little is known regarding the diversity and potential impact of helminths and arthropod parasites on marine mammal health, despite being one of the areas of the world with the highest diversity of marine vertebrates (Bakun and Week 2008; Gómez-Puerta and Gonzáles-Viera 2015; Gutierrez *et al.* 2016). Here, we characterize the tissue damage of helminth and arthropod parasites on Peruvian fur seals (PFS, *Arctocephalus australis*) and South American sea lions (SASL, *Otaria byronia*) from Punta San Juan, Peru, highlighting the effect of the most pathogenic species identified.

*Corresponding author: mseguel@uga.edu

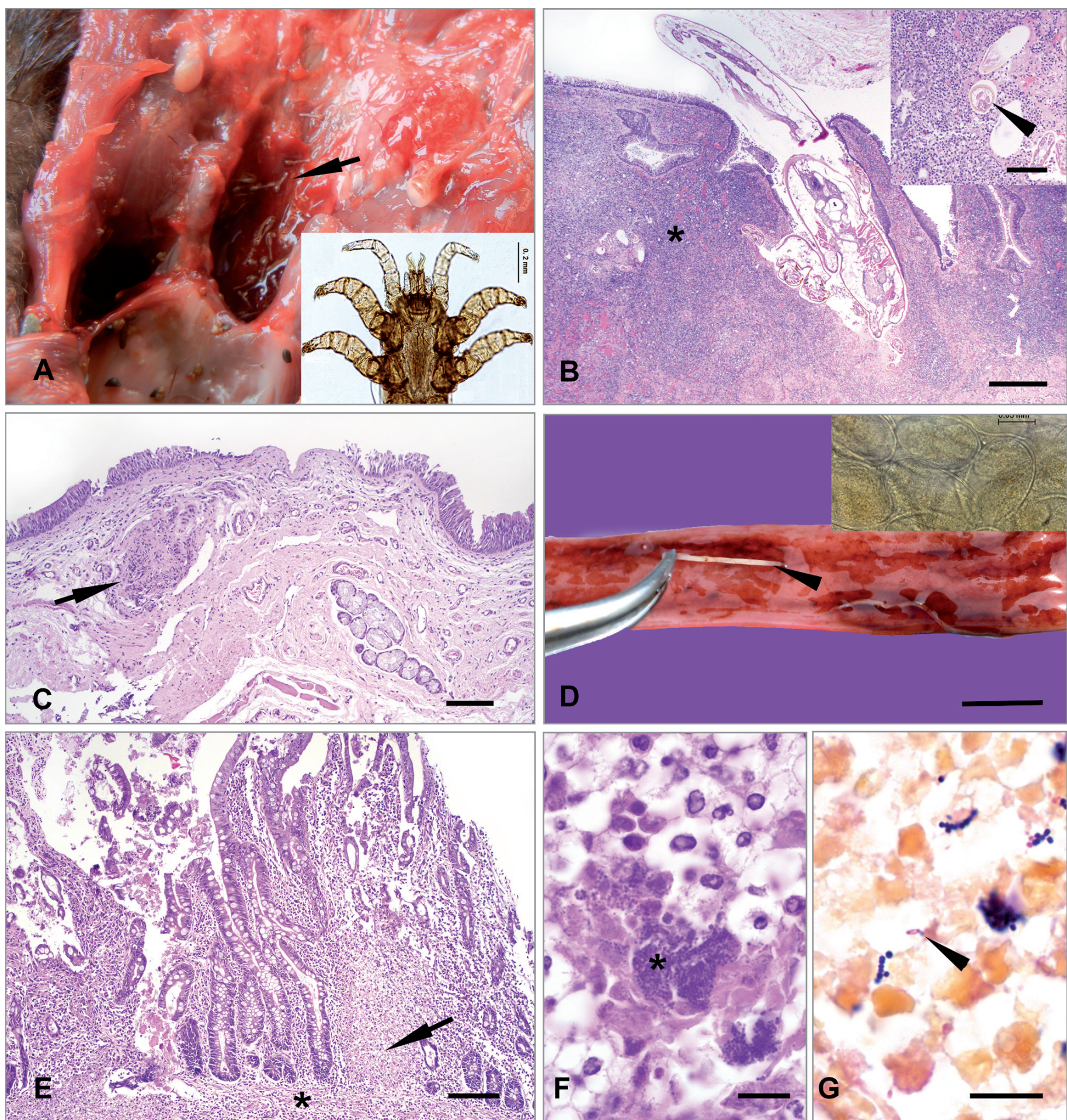


Fig. 1. Nasopharyngeal mite (*Orthohalarachne attenuata*) and hookworm (*Uncinaria sp.*) lesions in Peruvian fur seals (*Arctocephalus australis*) and South American sea lions (*Otaria byronia*) from Punta San Juan, Peru. **A.** – Mites attached to the nasopharyngeal mucosa of a subadult Peruvian fur seal (Arrow). Inset: Morphological details of *O. attenuata*. **B.** – Photomicrograph of a section of the tissue showed in A. Respiratory mites penetrate deep into the mucosa and submucosa and are surrounded by a dense inflammatory infiltrate (asterisk). Hematoxylin and eosin (H&E). Scale bar = 500 μ m. Inset, detail of inflammatory infiltrate surrounding rests of mites exoskeleton (arrow head). Scale bar = 100 μ m. **C.** – Photomicrograph of the nasopharynx of a South American sea lion parasitized with *O. attenuata*. There is mild erosion and ulceration of the epithelium and scant inflammatory infiltrate and fibrosis in the submucosa (arrow). H&E. Scale bar = 100 μ m. **D.** – Hookworm infection in a Peruvian fur seal pup. A large female hookworm is deeply embedded in the small intestine mucosa. In adjacent areas there are several hemorrhagic hookworm attachment sites (arrow head). Scale bar = 1 cm. Inset: Detail of *Uncinaria sp.* eggs within a female's uterus. **E.** – Photomicrograph of the small intestine showed in D. Intestinal villi are irregular and there is marked goblet cell hyperplasia. Deep in the mucosa are hookworm feeding tracks (arrow) and inflammatory infiltrate extent into the submucosa (asterisk). H&E. Scale bar = 100 μ m. **F.** – Higher magnification of a hookworm feeding track with numerous bacteria colonies (asterisk) surrounded by macrophages. H&E. Scale bar = 10 μ m. **G.** Gram stain of hookworm feeding track showed in G. There are rare gram-negative bacilli (arrow head) and occasional chains of gram-positive cocci. Scale bar = 5 μ m

Table I. Lesions caused by helminth and arthropod parasites in Peruvian fur seals (*Arctocephalus australis*) and South American sea lions (*Otaria flavescens*) at Punta San Juan, Peru

Case No	Species	Age class	Sex	Body condition	Parasite species	Burden	Parasite associated lesions	Cause of Death
1.	Fur seal (<i>A australis</i>)	Subadult	Female	Fair	<i>Orthohalarachne attenuata</i>	78	Marked, diffuse, hyperplastic, lymphoplasmacytic and histiocytic pharyngitis with pharyngeal glands hyperplasia and ectasia	Pneumonia
2.	Fur seal (<i>A australis</i>)	Subadult	Female	Fair	<i>Orthohalarachne attenuata</i> + <i>Corynosoma australe</i>	38 (Oa) 56 (Ca)	Moderate hyperplastic lymphoplasmacytic pharyngitis. Diffuse moderate edema.	Trauma
3.	Fur seal (<i>A australis</i>)	Pup	Male	Good	<i>Uncinaria sp</i>	101	Moderate to marked, diffuse, lymphoplasmacytic and eosinophilic enteritis with goblet cell hyperplasia and hookworm feeding tracks.	Trauma
4.	Fur seal (<i>A australis</i>)	Pup	Female	Poor	<i>Uncinaria sp</i>	21	Moderate to marked, diffuse, lymphoplasmacytic and eosinophilic enteritis with goblet cell hyperplasia and deep hookworm feeding tracks.	Starvation
5.	Fur seal (<i>A australis</i>)	Pup	Male	Poor	<i>Uncinaria sp</i>	121	Marked, diffuse, lymphoplasmacytic and histiocytic enteritis with goblet cell hyperplasia, deep hookworm feeding tracks and coccobacilli colonies.	HEB
6.	Fur seal (<i>A australis</i>)	Pup	Female	Poor	<i>Uncinaria sp</i>	60	Moderate, diffuse, lymphoplasmacytic and histiocytic enteritis with goblet cell hyperplasia, deep hookworm feeding tracks and coccobacilli colonies.	Starvation
7.	Fur seal (<i>A australis</i>)	Pup	Male	Good	<i>Uncinaria sp</i>	118	Marked, diffuse, lymphoplasmacytic and histiocytic enteritis with goblet cell hyperplasia, deep hookworm feeding tracks and coccobacilli colonies.	HEB
8.	Fur seal (<i>A australis</i>)	Pup	Male	Good	<i>Uncinaria sp</i>	90	Moderate, lymphoplasmacytic and eosinophilic enteritis with goblet cell hyperplasia and hookworm feeding tracks.	Trauma
9.	Fur seal (<i>A australis</i>)	Pup	Female	Fair	<i>Orthohalarachne attenuata</i>	41	Mild purulent pharyngitis	Trauma
10.	Sea lion (<i>O flavescens</i>)	Adult	Female	Good	<i>Orthohalarachne attenuata</i>	61	Minimal diffuse hyperplastic and lymphoplasmacytic pharyngitis with mild multifocal stroma elastosis.	Trauma
11.	Sea lion (<i>O flavescens</i>)	Adult	Male	Good	<i>Corynosoma australe</i>	300	Diffuse moderate edema.	Unknown
12.	Sea lion (<i>O flavescens</i>)	Adult	Male	Good	<i>Corynosoma australe</i>	210	Mild, diffuse, lymphoplasmacytic colitis.	Trauma
13.	Sea lion (<i>O flavescens</i>)	Adult	Male	Good	<i>Orthohalarachne attenuata</i> + <i>Contrac aecum osculatum</i>	31(Oa) 80 (Co)	Minimal diffuse hyperplastic and lymphoplasmacytic pharyngitis with mild multifocal stroma elastosis (Oa)	Unknown

14.	Sea lion (<i>O. flavescens</i>)	Subadult	Male	Good	<i>Corynosoma australe</i>	46	Diffuse moderate edema.	Trauma
15.	Sea lion (<i>O. flavescens</i>)	Subadult	Male	Good	<i>Corynosoma australe</i>	58	Diffuse moderate edema.	Trauma
16.	Sea lion (<i>O. flavescens</i>)	Pup	Female	Fair	<i>Orthohalarachne attenuata</i>	43	Minimal catarrhal pharyngitis	Trauma

HEB = Hookworm enteritis and bacteremia

Materials and Methods

In the austral summer of 2014, SASL (n = 8) and PFS (n = 32) were found dead at Punta San Juan, a site of the System of Islands, Islets and Guano Concentration Areas Protected Area (15°22'S, 75°11.5'W). Necropsies were performed in the field following a standard protocol (Dierauf 1994). During necropsies all helminth and arthropod parasites were collected recording the specific anatomic location. Sections of lung, nasopharynx, tonsil, bronchial lymph nodes, liver, kidney, small and large intestines, adrenal gland, spleen and all tissues from where parasites were collected or where parasites were still embedded in the tissue were placed in 10% buffered formalin and routinely processed for histopathology. The cause of death in each animal was determined based on gross and histologic findings following published diagnostic criteria for otariids (Seguel *et al.* 2011). All metazoan parasites identified at necropsy were placed in 70% ethanol, cleared with lactophenol (helminths) or potassium hydroxide (mites) mounted in glass slides with Canada balsam, measured and photographed for identification using standard parasitology keys (Banks 1910; Baylis 1933, Doestchman 1944; 1947, Lichtenfels 1980; Willmott and Chabaud 2009; Van Cleave 1945). Voucher specimens were placed in the collection of the laboratory of Fauna Silvestre y Zoonosis, Universidad Nacional Mayor de San Marcos, Lima, Peru.

Results

Thirty-two PFS, including 18 male, 14 female, and 24 pups, 8 juvenile/subadults were examined. The cause of death in these animals included blunt trauma to the head (3PFS and 4 SSL) and chest (1 PFS and 1 SSL), starvation (2 PFS), hookworm enteritis and bacteremia (HEB) (2 PFS), bronchopneumonia (1 PFS) and in 1 PFS and 1 SSL the cause of death could not be determined (Table I). Nine PFS (28%) had helminth and/or arthropod parasites. In the case of SASL 6 out of the 8 (75%) animals examined (7 males, 1 female, 1 pup, 7 subadults) had metazoan parasites. The demographic information, parasite species, burden, most significant lesions and most likely cause of death of the animals with parasitic infections are summarized in Table I. In PFS, respiratory mites (*Orthohalarachne attenuata*) were deeply embedded in the nasopharyngeal mucosa and caused moderate to marked lymphoplasmacytic and histiocytic pharyngitis with epithelial and pharyngeal mucous

gland hyperplasia (Fig. 1A and 1B). One PFS had occasional small areas of necrosis deep in the mucosa associated with rests of mites and small colonies of Gram-positive cocci. This animal died as consequence of moderate and diffuse histiocytic and neutrophilic bronchopneumonia with presence of small numbers of macrophages and neutrophils in alveolar septae. In SASLs, mite attachment was more superficial and associated with occasional epithelial erosions, mild mucosa hyperplasia, mild fibrosis and infiltration of rare lymphocytes and plasma cells (Fig. 1C). Hookworms (*Uncinaria sp.*), were the most common parasite in PFS pups (Table I) (Fig. 1D). In three animals between 60 and 118, 1–2 cm in length hookworms admixed with abundant hemorrhagic intestinal content occupied the distal jejunum and ileum. In these animals, skeletal muscles and mucus membranes were moderately pale and blood moderately aqueous. Histologically, these animals had moderate to marked lymphoplasmacytic and histiocytic enteritis with goblet cell hyperplasia and numerous hookworm feeding tracks in the submucosa (Fig. 1E). Feeding tracks usually contained colonies of gram-negative coccobacilli and gram-positive cocci (Fig. 1F and 1G). Two of these pups were considered to have died due to "hookworm enteritis and bacteremia", while the other pup died most likely due to starvation and hookworm lesions. In three pups, hookworms caused moderate to marked lymphoplasmacytic and eosinophilic enteritis with marked goblet cell hyperplasia. In these pups, hookworm feeding tracks were confined to the mucosa and bacterial translocation to the submucosa was not observed. Additionally, one of these pups had occasional coccidian merozoites within apical enterocytes. Moderate numbers (46 to 300 specimens) of the acanthocephalan *Corynosoma australe* were found in in the small intestine of one subadult PFS and five adult SASL. There were no major tissue changes associated with the presence of this parasite, besides mild to moderate edema at the site of attachment. The nematode *Contracaecum osculatum* was found in moderate numbers (80 specimens) in the stomach of one adult SASL, and no associated lesions were observed.

Discussion

The number of PFS with parasites (28%) is low if compared with other populations of fur seals, where nematode or mite infection can affect up to 100% of the population (Kim *et al.* 1980; Seguel *et al.* 2017). The number of sampled SASL is

low to evaluate the overall prevalence of parasitic infections in this population but our preliminary data suggests it could be higher than in fur seals. The reason of the relative low prevalence of parasitic infection in PFS could be related to the age class of the sampled animals which were mostly pups. Parasitic infections in this fur seal age class are limited to hookworms and respiratory mites because they only feed on their mother's milk. Therefore, only parasites transmitted through the milk (hookworms) or through direct contact (mites) can infect these animals, which contrast with SASL which were infected mostly by parasite transmitted through fish. Additionally, these parasitic infections frequency tend to decrease with low host density. PFS are in lower numbers compared to their historic abundance at Punta San Juan, Peru (Cárdenas-Alayza and Oliveira 2016), being one of the factors that could be associated with low prevalence of parasites in this population.

There was a marked difference between SASL and PFS in the severity of the inflammatory reaction against respiratory mites. PFS tended to have more inflammation and tissue damage associated to these parasites. This difference could be related to the deeper location of mites within the PFS tissues or correspond to animal age and/or temporal variation in the infection process. Lesions observed in fur seal pups may be more recent infections in relatively naïve animals compared to those noted in SASL, which were mostly subadult and adult animals. The effect of parasite burden is probably less significant, as both species had similar parasite loads. The role of *O. attenuata* in causing disease in pinnipeds is not clear, as reports of significant health effects are rare and related to extremely heavy infections (Dunlap and Piperlij 1976; Kim *et al.* 1980). However, in this report, one PFS had significant lesions associated with secondary invasion of bacteria, which could have contributed to the development of bronchopneumonia and eventual death.

The lesions caused by *Uncinaria sp.* in PFS are identical to those described in South American fur seals at the Chilean Patagonia infected with the same *Uncinaria sp.* (Seguel *et al.* 2017). In otariid pups, hookworms feed deep into the submucosa generating tracks that are infected by enteric bacteria, with the subsequent translocation of bacteria or toxins into the bloodstream (Seguel *et al.* 2017). In this study, although blood cultures were not performed, we called the cause of death "hookworm enteritis with bacteremia" since this term describes the aforementioned syndrome (Spraker *et al.* 2007; Seguel *et al.* 2017). Although the overall prevalence of this parasite was low (6/32), its presence and the level of tissue damage caused suggest that could become a significant pathogen if the environmental conditions for its transmission change (e.g. increase in host density). Hookworms are the most significant parasitic disease of otariids and in some populations can kill more than 30% of pups born each year (Seguel and Gottdenker 2017). The pathogenic potential of these nematodes is highlighted by this study since animals dying due to hookworm enteritis syndrome had less than 150 nematodes in the

intestine, a burden considered low if compared to other fur seal populations with median burdens of ~ 300 nematodes per pup (Seguel *et al.* 2017, Seguel and Gottdenker 2017). However, this low burden was enough to cause substantial tissue damage, and associated to secondary bacterial infection, kill some pups.

The lack of significant lesions associated with *C. australe* infection, despite a moderate parasite burden in several animals, suggests SASL and PFS have developed tolerance to this parasite. For *C. osculatum*, the observed case had a low burden, which probably accounted for the lack of associated lesions.

The role of metazoan parasites as agents of disease in SASLs is not clear, as the lack of significant lesions in our study could be associated with a small sample size and overrepresentation of adult males. However, *O. attenuata* and *Uncinaria sp.* have the potential to cause disease and mortality in PFS. In the last century, the PFS population suffered a dramatic decline and continues to face significant conservation challenges due to reduced genetic diversity, isolation, small population size and vulnerability to environmental change (Cárdenas-Alayza *et al.* 2016). Therefore, further evaluation of the population effect, transmission dynamics and factors limiting/favoring respiratory mites and hookworm infection in PFSs is warranted.

Acknowledgements. Mauricio Seguel was supported by a Morris Animal Foundation fellowship (Grant N D16ZO-413). Sample collection at Punta San Juan - RNSIIPG was authorized by research permit RJ No.09-2013-SERNANP-RNSIIPG, and funded by Chicago Zoological Society's Chicago Board of Trade Endangered Species Fund. We gratefully acknowledge support from SERNANP and facility access by Agrorural at Punta San Juan

References

- Cárdenas-Alayza S., Oliveira L. 2016. *Arctocephalus australis* (Peruvian/Northern Chilean subpopulation). The IUCN Red List of Threatened Species 2016: e.T72050476A72050985. <http://dx.doi.org/10.2305/IUCN.UK.2016-1.RLTS.T72050476A72050985.en>. Downloaded on 03 October 2017
- Cárdenas-Alayza S., Oliveira L., Crespo E. 2016. *Arctocephalus australis*. The IUCN Red List of Threatened Species 2016: e.T2055A45223529. <http://dx.doi.org/10.2305/IUCN.UK.2016-1.RLTS.T2055A45223529.en>. Downloaded on 26 April 2017
- Banks N. 1910. New American Mites (Arachnoidea, Acarina). *Proceedings Entomological Society of Washington*, 12, 2–12
- Baylis H.A. 1933. A new species of the nematode genus *Uncinaria* from a sea-lion, with some observations on related species. *Parasitology*, 25, 308–316
- Bakun A. and Weeks S.J., 2008. The marine ecosystem off Peru: What are the secrets of its fishery productivity and what might its future hold? *Progress in Oceanography*, 79, 290–299
- Dierauf L. 1994. Pinniped forensic necropsy and tissue collection guide. First. Washington: National Oceanic and Atmospheric Administration; pp. 80
- Dunlap J.S. and Piperlij R.C. 1976. Lesions Associated with *Orthohalarachne attenuata* (Halarachnidae) in the Northern fur

- seal (*Callorhinus ursinus*). *Journal of Wildlife Disease*, 12, 42–44.
- Doetschman W.H. 1944. A new species of endoparasitic mite of the family Halarachnidae (Acarina). *Transactions of the American Microscopical Society*, 63, 68–72
- Gomez-Puerta L. and Gonzales-Viera O. 2015. Ectoparasites from the South American sea lion (*Otaria flavescens*) from Peruvian coast. *Revista Peruana de Biología*, 22, 259–262
- Gulland F.M. and Hall A.J. 2007. Is marine mammal health deteriorating? Trends in the global reporting of marine mammal disease. *EcoHealth*, 4, 135–150
- Gutierrez D., Akester M., Naranjo L. 2016. Productivity and sustainable management of the Humboldt current large marine ecosystem under climate change. *Environmental Development*, 17, 126–144
- Kim K.C., Haas V.L., Keyes M.C. 1980. Populations, microhabitat preference and effects of infestation of two species of Orthohalarachne (Halarachnidae: Acarina) in the northern fur seal. *Journal of Wildlife Diseases*, 16, 45–51
- Lichtenfels J.R. 1980. Keys to genera of the superfamilies Ancylostomatoidea and Diaphanocephaloidea. In: Anderson, R. C., Chabaud, A.G. & Willmott, S. (Eds) CIH Keys to the Nematode Parasites of Vertebrates, No. 8. Farnham Royal, Bucks, UK: Commonwealth Agricultural Bureaux, pp. 1–41
- Seguel M. and Gottdenker N. 2017. The Diversity and Impact of hookworm infections in wildlife. *International Journal of Parasitology: Parasites and Wildlife*, 6, 177–194
- Seguel M., Muñoz F., Navarrete M.J., Paredes E., Howerth E., Gottdenker N. 2017. Hookworm Infection in South American Fur Seal (*Arctocephalus australis*) Pups. *Veterinary Pathology*, 54, 288–297
- Seguel M., Paredes E., Pavés H., Molina R., Henríquez, F., De Groote F., Schlatter R. 2011. Pathological findings in South American fur seal pups (*Arctocephalus australis gracilis*) found dead at Guafo Island, Chile. *Journal of Comparative Pathology*, 145(2–3), 308–17.
- Sepúlveda M.A., Seguel M., Alvarado-rybak M., Muñoz-zanzi C., Tamayo R. 2015. Postmortem Findings in Four South American Sea Lions (*Otaria byronia*) from an Urban Colony. *Journal of Wildlife Disease*, 51, 279–282
- Spraker T.R., DeLong R.L., Lyons E.T., Melin S.R. 2007. Hookworm enteritis with bacteremia in California sea lion pups on San Miguel Island. *Journal of Wildlife Diseases*, 43, 179–88
- Van Cleave H. 1945. The genital vestibule and its significance in the morphology and taxonomy of the Acanthocephala, with particular reference to the genus *Corynosoma*. *Journal of Morphology*, 77, 299–315

Received: October 13, 2017

Revised: May 2, 2018

Accepted for publication: July 3, 2018